IN THE UNITED STATES PATENT AND TRADE MARK OFFICE

Serial No :	10/030,147	) Examiner:
Inventors :	Ailsa Helen CRAIG et al	) Jyothsna A. Venkat
Filed :	May 20, 2002	)
For :	HAIR CARE COMPOSITION	)

DECLARATION OF STEWART PAUL LONG

I, STEWART PAUL LONG, of 22 Hilltop Drive, Oakham, Rutland, LE15 6NF, United Kingdom, declare as follows:

My Background and Experience

1. I hold a Master of Science (M.Sc.) degree in Biochemistry from the University of Liverpool.

2. After graduation from university, I worked for approximately eight years for Unilever PLC, conducting research on skincare and haircare products. In 1998 I joined The Boots Company PLC where I now hold the position of Scientific Adviser (Biotechnology and Haircare).

3. In my work at Boots I have gained extensive experience of the development of research methodologies concerned with haircare products, and in the use of anti-oxidants in such products. I am one of the inventors of the present application.

Introduction

4. Until recently, the performance criteria upon which hair products were selected tended to be centred upon dealing with immediate needs (static problems, greasiness, over-dry hair etc) rather than protecting against long term damage. The consumer did not select hair products as 'insurance' against perceived long term damage from the UV content of sunlight in the way that they would choose a skincare product containing sunscreens to protect against the threat of premature skin ageing.

5. In the last few years, however, there have been major changes in the haircare market. It has become increasingly sophisticated, reflecting the research and development investments by the major companies into understanding the fundamentals of hair biochemistry and structure. Products now seek to address the long term damage caused by environmental factors such as UV light and pollution and actively strengthen hair to reduce long term mechanical damage (which manifests as poor aesthetics and more hair breakages). The consumer has responded positively to this investment in technology and product performance, increasingly choosing higher value, premium haircare products. The effects of the environment on hair condition are becoming understood by consumers and they are beginning to demand the same sort of performance from hair products as they do from their skincare regime. Haircare products now need to deliver significant long-term benefits, whether it be protection against UV damage to coloured and natural hair or addressing the changing composition of hair as we age, as well as the immediate performance benefits that have always been expected.

6. In haircare, the effects of UV light have been considered largely with respect to the fading of artificially coloured hair, with a marked increase in the number of products on the market containing sunscreens. Less attention has been given to the deleterious effects of UV light on hair condition in general and technologies to reduce those effects.

7. The following experiments were carried out in our laboratories to investigate the effects of combinations of anti-oxidants, with a view to developing improved haircare products.

Methodologies

8. *In vitro* Tests

Linoleic acid (model hair lipid) was incubated in the presence of various anti-oxidants and anti-oxidant combinations and was exposed to broad spectrum UV-A / UV-B to induce oxidation of the lipid. Following extraction of the lipid into methanol, the amount of lipid hydroperoxides (free radical-generated damage) formed was measured with a specific colorimetric biochemical test. The degree of inhibition afforded by the anti-oxidants was thus measured and compared to irradiated controls.

9. *In vivo* Tests

The effects of solar simulated light and heat from styling products on lipid hydroperoxide formation, and the effects of combinations of anti-oxidants in inhibiting such effects, were investigated.

Virgin brown hair tresses were used for all experimental work.

In order to investigate the effects of antioxidants on lipid hydroperoxide formation, a single tress was halved and washed with a base formulation on one half and the base formulation with active ingredient on the other half. The washing regime involved applying 1ml of product to a wet hair tress of dry weight 0.75g and massaging for 2 mins. The tresses were then washed with running tap water for 30 sec. After air-drying the tresses and exposing to solar simulated light, lipids (not covalently bound) were extracted from approximately 1mg samples of the

tresses using 1ml of chloroform/methanol (2:1 v/v) for 2 hours in the dark, with tumbling. An aliquot of the extract was removed and evaporated under nitrogen gas to dryness. Samples were then resuspended in 50µl of methanol for assaying.

The levels of lipid hydroperoxide were assayed by conversion to lipid alcohols, catalysing formation of methylene blue from a colourless precursor.

Results

10. *In vivo* measurements

10.1 Compositions of four Examples taken from the specification of the present application were investigated and compared with control formulations that were identical save that the anti-oxidants were omitted.

The composition of Example 1 containing morus alba (0.2%), origanum vulgare (0.2%) and panax ginseng (0.2%) showed an amount of oxidised lipid of 113 nmol/ml, compared to 181 nmol/ml for the control. The reduction in the amount of oxidised lipid was therefore approximately 38%.

The composition of Example 8 containing morus alba (0.2%), origanum vulgare (0.2%) and panax ginseng (0.2%) showed an amount of oxidised lipid of 372 nmol/ml, compared to 553 nmol/ml for the control. The reduction in the amount of oxidised lipid was therefore approximately 33%.

The composition of Example 15 containing morus alba (0.2%), origanum vulgare (0.2%) and panax ginseng (0.2%) showed an amount of oxidised lipid of 482 nmol/ml, compared to 731 nmol/ml for the control. The reduction in the amount of oxidised lipid was therefore approximately 34%.

The composition of Example 16 containing rosmarinus officinalis (0.2%), origanum vulgare (0.2%) and panax ginseng (0.2%) showed an amount of oxidised lipid of 601 nmol/ml, compared to 694 nmol/ml for the control. The reduction in the amount of oxidised lipid was therefore approximately 13%.

10.2 Effect of solar simulated light on lipid hydroperoxide formation

Hair tresses were exposed to solar simulated light for periods up to 24 hours. Lipid hydroperoxides were extracted and assayed as described above. A linear relationship until at least 16 hours irradiation was observed, as shown in Figure 1. For subsequent experiments comparing product effects, an irradiation time of 8 hours was used, as this gave sufficient sensitivity and represented around 24 hours of constant solar equivalent irradiation (approx. 3 full days clear sky irradiation).

10.3 Effect of heat styling on lipid hydroperoxide formation

The excessive use of heat styling appliances such as hairdryers and tongs is well known to lead to a deterioration in hair condition. The hair becomes brittle, weaker and rough to the touch after prolonged and repeated use of heat styling products and there is a marked reduction in hair shine. We wanted to investigate whether single episodes of heat drying of hair tresses caused changes in lipid hydroperoxide formation, as we considered free radical mediated events to be possibly implicated in initiating damage. Hair tresses were washed with shampoo and either allowed to air-dry naturally, or dried using various heating regimes.

The effects of varying the distance from the hairdryer to the tress and of the drying time are shown in Figure 2.

It can be seen that all treatments lead to elevated levels of lipid hydroperoxides, compared to the control which was dried naturally, and that decreasing the distance to the tress from 10cm to 5cm has a marked effect on the oxidation damage induced. We also observed that brushing the hair whilst drying had no effect on the degree of damage induced, whereas the use of tongs on the hair markedly increased damage, compared to heat drying alone.

10.4 Effect of antioxidants on reducing the oxidation damage caused by heat styling

Having shown that heat styling can lead to an increased level of lipid peroxidation within hair, we investigated whether antioxidant containing natural plant extracts would prove effective in reducing this damage. Shampoo formulations with and without a complex of 0.2% (w/w) of each of Morus alba, Panax ginseng and Origanum vulgare (0.6% w/w total extracts) were tested using the method described above, except a more aggressive heat drying regime of 10mins heating from a distance of 5cm was used. The formulation with the antioxidant complex afforded significant and substantial protection from this particularly harsh test, reducing the oxidation damage measured by over 72% ($p < 0.01$), as shown in Figure 3.

10.5 Effect of antioxidants on solar simulated light induced lipid hydroperoxides

Hair tresses were washed using either a base shampoo formulation, or with a corresponding formulation containing a complex of 0.2% (w/w) of each of Morus alba, Panax ginseng and Origanum vulgare (0.6% w/w total extracts). After rinsing and air drying, the tresses were exposed to solar simulated light and the levels of lipid hydroperoxides determined. The formulation with the antioxidant complex afforded significant protection, reducing the oxidation damage measured by over 64% ($p < 0.01$), as shown in Figure 4.

11. *In vitro* Measurements

11.1 The measured percentage inhibition of free radical-mediated lipid peroxidation when compared to the control formulation alone is given below for individual anti-oxidants and combinations of anti-oxidants:

Anti-oxidant	% inhibition
Morus alba (ma)	0
Origanum vulgarum (ov)	49
Panax ginseng (pg)	5.7
Sodium ascorbyl phosphate (nap)	21
Grape seed (gs)	0
Camellia sinensis (cc)	19
Observed effect of nap/pg/ov	100
Predicted effect of nap/pg/ov	75.7
Observed effect of nap/ma/ov	100
Predicted effect of nap/ma/ov	70
Observed effect of nap/ma/cc	100
Predicted effect of nap/ma/cc	40
Observed effect of nap/ma/gs	81
Predicted effect of nap/ ma/gs	21

Thus it can be seen that the observed inhibiting effect of using the above combinations of anti-oxidants is greater than that which would be expected from the sum of the contributions of the individual anti-oxidants.

11.2 In a second series of experiments using anti-oxidants collected in a later season, the following results were obtained:

Anti-oxidant	% inhibition
Morus alba (ma)	37
Origanum vulgarum (ov)	29
Rosmarinus officinalis (ro)	15
Panax ginseng (pg)	10
Observed effect of ro/pg/ov	88
Predicted effect of ro/pg/ov	54
Observed effect of pg/ma/ov	93
Predicted effect of pg/ma/ov	76

Again, it can be seen that the observed inhibiting effect of using the above combinations of anti-oxidants is greater than that which would be expected from the sum of the contributions of the individual anti-oxidants.

11.3 In a further series of measurements, the following series of results were obtained for individual anti-oxidants, expressed as the % inhibition of free radical mediated lipid peroxidation compared to that achieved using the vehicle alone.

Antioxidant	% inhibition
Morus alba (m)	0
Origanum vulgare (or)	29
Rosmarinus officinalis (r)	15
Panax ginseng (gin)	5.7
Grape seed (gs)	0
Ascorbyl phosphate (ap)	49
Ascorbic acid ester (aae)	48

The results obtained for combinations of three antioxidants (at the same total antioxidant concentration as for the individual antioxidants) are shown, in comparison with the individual components, in Figures 5, 6 and 7. These show synergistic effects for the following combinations of anti-oxidants:

(Figure 5) Rosmarinus officinalis / Panax ginseng / Ascorbyl phosphate
Panax ginseng / Morus alba / Ascorbyl phosphate
Rosmarinus officinalis / Morus alba / Ascorbyl phosphate

(Figure 6) Rosmarinus officinalis / Oreganum vulgarum / Grape seed
Morus alba / Oreganum vulgarum / Panax ginseng
Grape seed / Oreganum vulgarum / Panax ginseng
Rosmarinus officinalis / Oreganum vulgarum / Panax ginseng

(Figure 7) Ascorbyl phosphate / Oreganum vulgarum / Morus alba
Panax ginseng / Oreganum vulgarum / Ascorbyl phosphate
Oreganum vulgarum / Ascorbic acid ester / Panax ginseng
Ascorbyl phosphate / Oreganum vulgarum / Rosmarinus officinalis
Ascorbic acid ester / Oreganum vulgarum / Morus alba
Grape seed / Ascorbyl phosphate / Morus alba
Ascorbic acid ester / Grape seed / Rosmarinus officinalis

Further measurements were made, in a separate series of experiments, and the following results were obtained:

Combination	Predicted % Inhibition	Actual % Inhibition
ap/m/or	78	100
ap/m/gin	54.7	98.6
ap/m/gs	49	81
ap/gin/r	69.7	92
ap/m/r	64	99.9
or/m/gin	34.7	93
or/gin/gs	34.7	100
r/or/gin	49.7	88
ap/or/gin	69.7	100
or/aae/gin	48	88
aae/gs/r	63	94

Conclusions

12. The results described above show that lipid peroxidation is a consequence of exposure of hair to sunlight and to the heat associated with hair styling. The results further show that such peroxidation can be inhibited by various anti-oxidants.

13. Surprisingly, the results show that combinations of three anti-oxidants selected from:

(a) ascorbic acid, its salts and esters;
(b) morus alba;
(c) origanum vulgare;
(d) panax ginseng;
(e) rosmarinus officinalis;
(f) camellia sinensis; and
(g) grape seed extract;

are synergistic in that the effect of such combinations is greater than would be predicted on the basis of the effects of the individual anti-oxidants alone. This has been demonstrated for all of the following combinations of anti-oxidants:

Rosmarinus officinalis / Panax ginseng / Ascorbyl Phosphate
Panax ginseng / Morus alba / Ascorbyl phosphate
Rosmarinus officinalis / Morus alba / Ascorbyl phosphate
Morus alba / Origanum vulgare / Panax ginseng
Grapeseed / Origanum vulgare / Panax ginseng
Rosmarinus officinalis / Origanum vulgare / Panax ginseng
Ascorbyl Phosphate / Origanum vulgare / Morus alba
Panax ginseng / Origanum vulgare / Ascorbyl Phosphate
Origanum vulgare / Ascorbic acid ester / Panax ginseng
Ascorbyl phosphate / Morus alba / Grape seed
Ascorbic acid ester / Grape seed / Rosmarinus officinalis
Rosmarinus officinalis / Origanum vulgare / Grape seed
Ascorbyl phosphate / Origanum vulgare / Rosmarinus officinalis
Ascorbic acid ester / Origanum vulgare / Morus alba

14. In view of the fact that a synergistic effect has been demonstrated for a substantial proportion of all possible combinations of anti-oxidants covered by the claims of the present application, and in view of the fact that all of the anti-oxidants listed in the claims of the application occur in at least one of the combinations for which synergy has been demonstrated, I believe it is reasonable to suppose that other combinations falling within the scope of the claims would exhibit similar properties.

15. I do not believe that the synergistic effects described herein could have been predicted from the properties of the individual anti-oxidants or from the prior art.

Figure 1

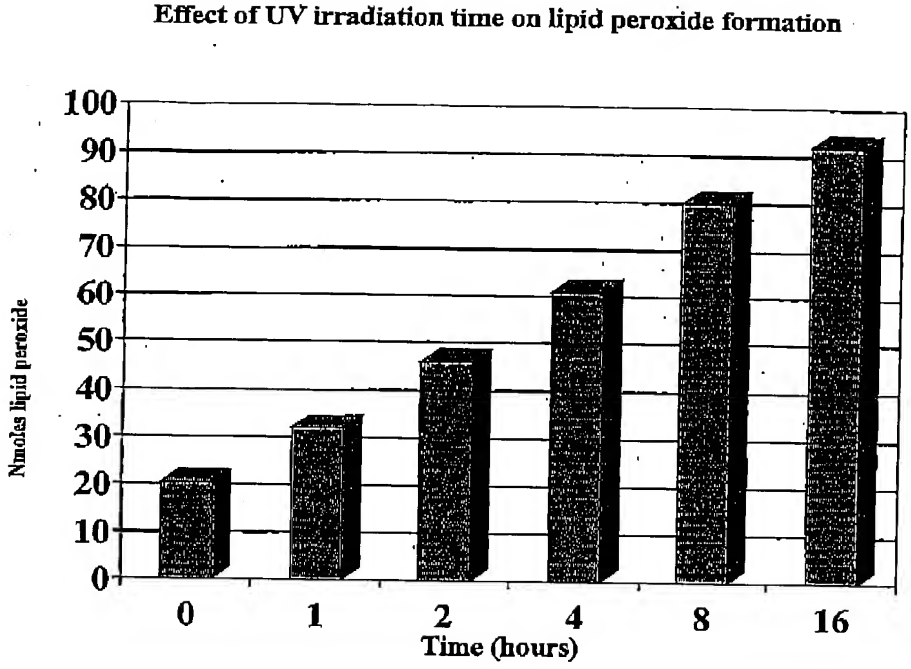

Effect of UV irradiation time on lipid peroxide formation
Nmoles lipid peroxide
100
90
80
70
60
50
40
30
20
10
0
0
1
2
4
8
16
Time (hours)

Figure 2

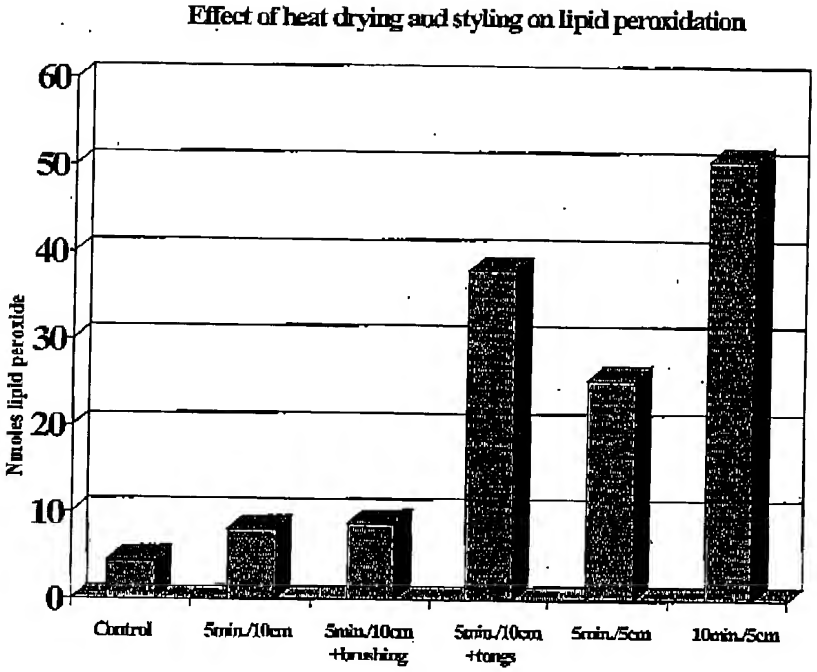

Effect of heat drying and styling on lipid peroxidation
Nmoles lipid peroxide
60
50
40
30
20
10
0
Control
5min./10cm
5min./10cm +brushing
5min./10cm +tongs
5min./5cm
10min./5cm

Figure 3

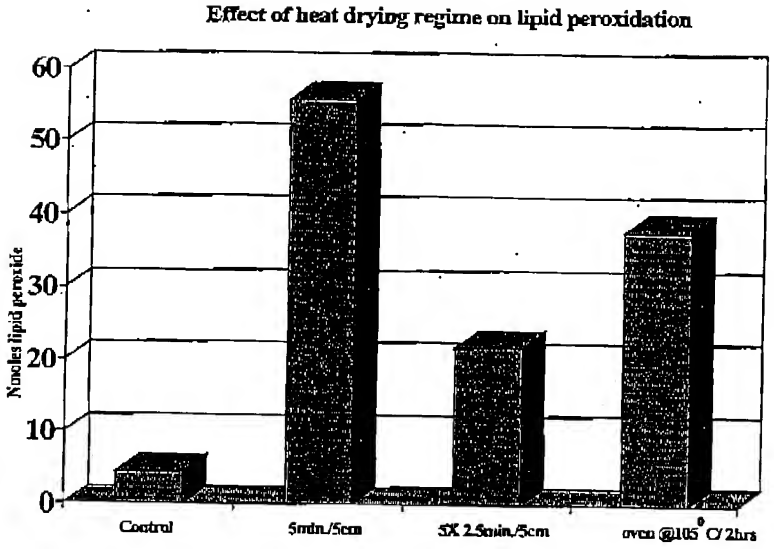

Effect of heat drying regime on lipid peroxidation
Nmoles lipid peroxide
60
50
40
30
20
10
0
Control
5mtn./5cm
5X 2.5min./5cm
oven @105° C/ 2hrs

Figure 4

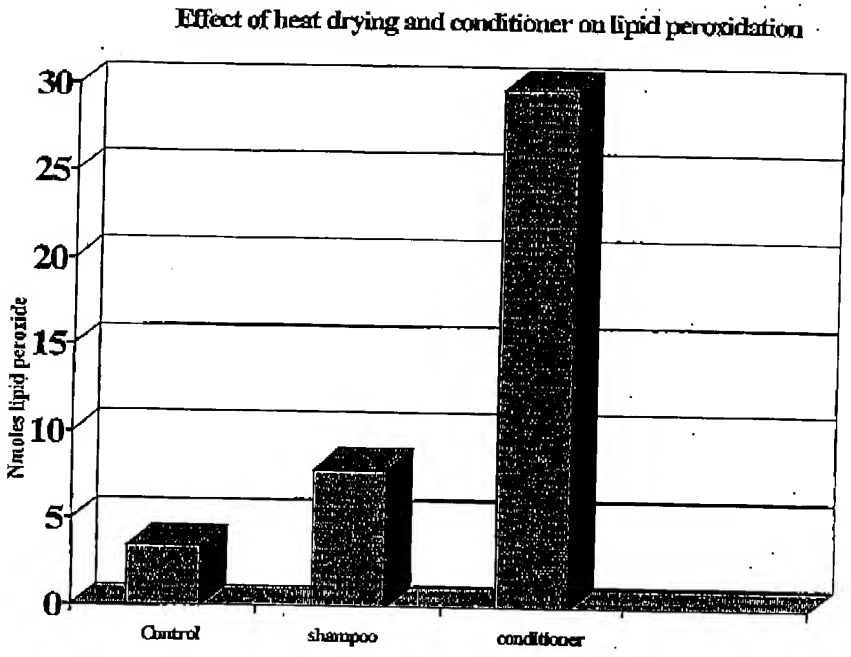

Effect of heat drying and conditioner on lipid peroxidation
Nmoles lipid peroxide
30
25
20
15
10
5
0
Control
shampoo
conditioner

Figure 5

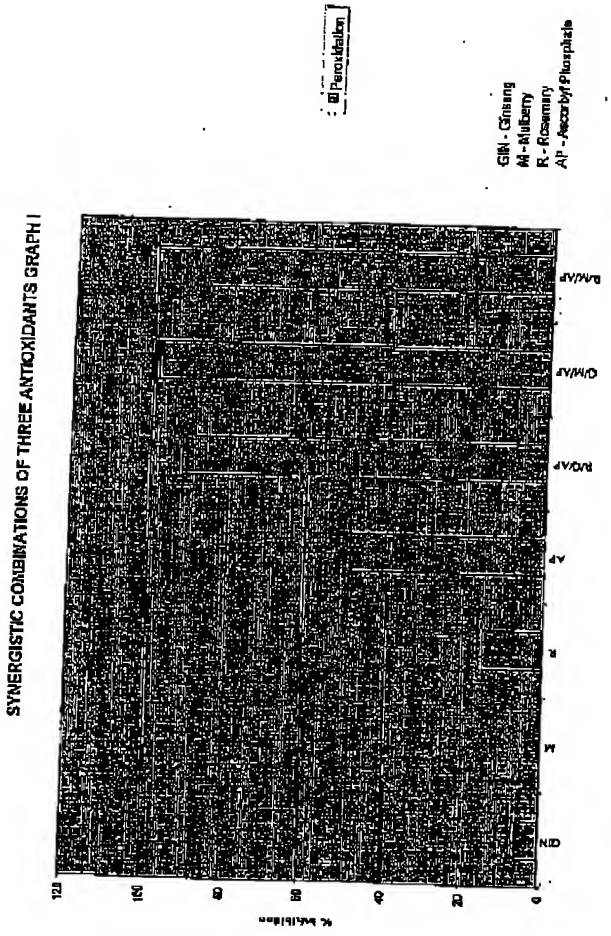

SYNERGISTIC COMBINATIONS OF THREE ANTIOXIDANTS GRAPH I
Peroxidation
GIN - Ginseng
M - Mulberry
R - Rosemary
AP - Ascorbyl Phosphate
G/M/R/AP
G/M/AP
R/G/AP
AP
R
M
GIN
120
100
80
60
40
20
0
% Inhibition

Figure 6

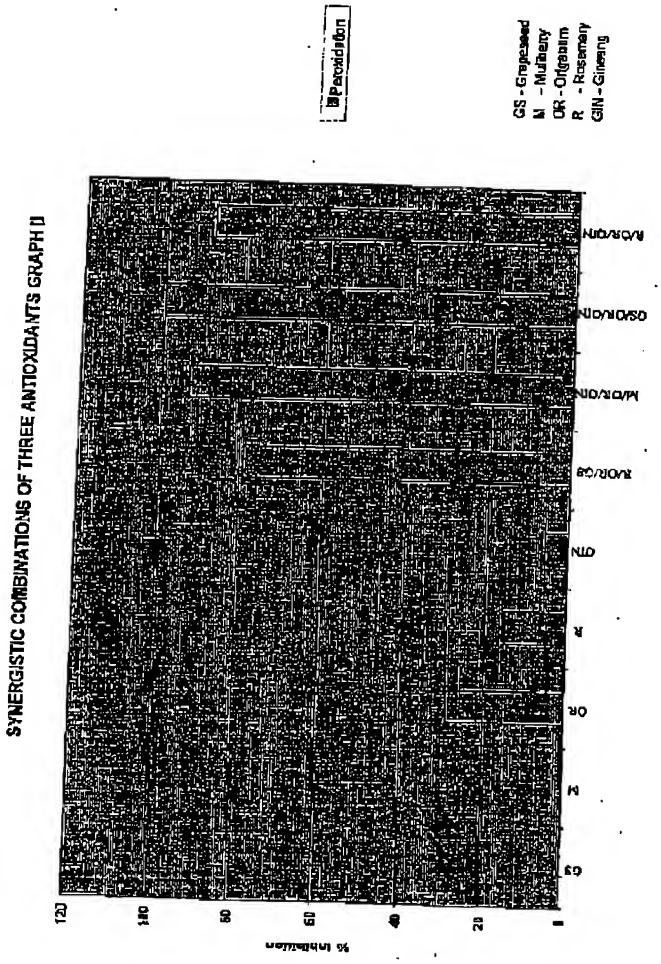
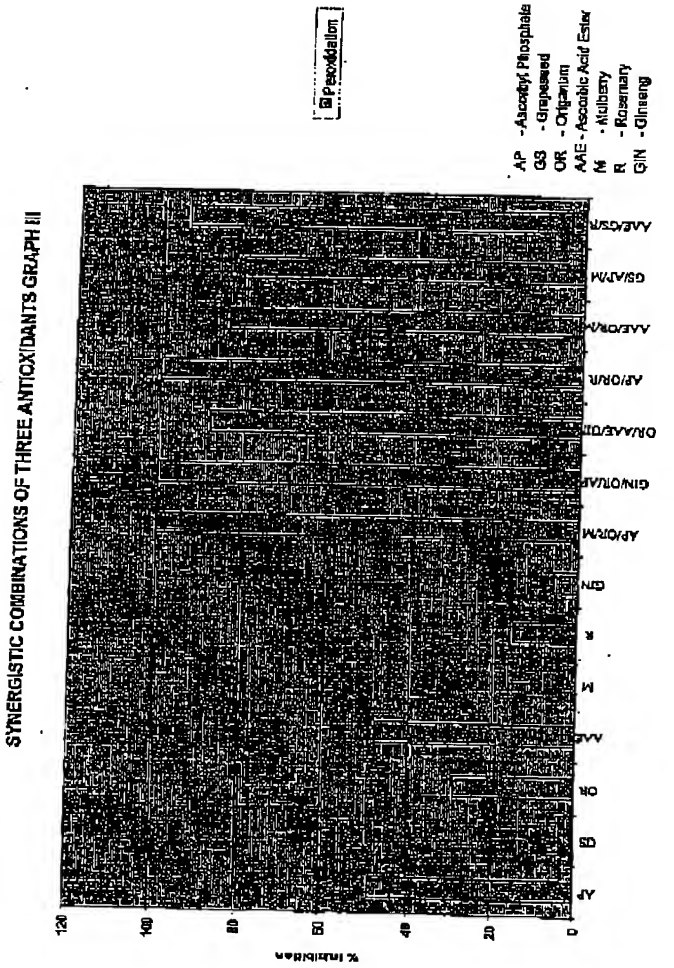

SYNERGISTIC COMBINATIONS OF THREE ANTIOXIDANTS GRAPH II
Peroxidation
GS - Grapeseed
M - Mulberry
OR - Origanum
R - Rosemary
GIN - Ginseng
GS
M
OR
R
GIN
R/OR/GS
M/OR/GIN
GS/OR/GIN
R/GS/GIN
% Inhibition
120
100
80
60
40
20
0

Figure 7


SYNERGISTIC COMBINATIONS OF THREE ANTIOXIDANTS GRAPH III
Peroxidation
AP - Ascorbyl Phosphate
GS - Grapeseed
OR - Origanium
AAE - Ascorbic Acid Ester
M - Mulberry
R - Rosemary
GIN - Ginseng
% Inhibition
120
100
80
60
40
20
0
AP
GS
OR
AAE
M
R
GIN
AP/OR/M
GIN/OR/AP
OR/AAE/GIN
AP/OR/R
AAE/OR/M
GS/AP/M
AAE/GS/R

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signed : _____

Stewart Paul LONG

Date : 26/7/03